

## Harm Reduction Methods

### I. Acceptability guidelines/approaches

- a) Carmines, E.L., "Evaluation of the potential effects of ingredients added to cigarettes. Part 1: Cigarette design, testing approach, and review of results". *Food Chem. Toxicol.* **40**: 77-91; 2002.
- b) Carchman, R., "Identification of 'animal or organism-based' short-term tests which are available for the assessment of the potentially harmful properties of tobacco smoke", *Report of Canada's expert committee on cigarette toxicity reduction*, Conference proceedings and Invited Papers, Appendix, page 41, 1998. [Prototype]
- c) Patskan, G.; Hsu, F.; Meisgen, T.; Stabbert, R.; Vanscheeuwijck, P.; Veltel, D., "Toxicological characterization of a novel cigarette paper". *Toxicol. Sci.* **54**: 398; 2000.

### II. Harm reduction guidelines

### III. Identification of targets for harm reduction

- a) Borgerding, M.F.; Bodnar, J.A.; Chung, H.L.; Mangan, P.P.; Morrison, C.C.; Risner, C.H.; Rogers, J.C.; Simmons, D.F.; Uhrig, M.S.; Wendelboe, F.N.; Wingate, D.E.; Winkler, L.S., "Chemical and biological studies of a new cigarette that primarily heats tobacco. Part 1. Chemical composition of mainstream smoke". *Food Chem. Toxicol.* **36**: 169-182; 1997.
- b) Fowles, J.; Bates, M.; "The chemical constituents in cigarettes and cigarette smoke: Priorities for harm reduction". A report to the New Zealand Ministry of Health. March 2000. [Online]: <http://www.ndp.govt.nz/tobacco/tobaccochem.pdf>. Accessed February 5, 2002.
- c) Haussmann, H.J.; Rustemeier, K.; Elves, R.G., "The use of risk analysis in selecting cigarette smoke compounds for reduction", Presented at the annual meeting of the Society of Risk Analysis, Seattle, Washington, December 2-5, 2001.
- d) International Agency for Research on Cancer. *IARC Monographs on the evaluation of the carcinogenic risk of chemicals to humans: Tobacco Smoking, Volume 38*. International Agency for Research on Cancer, World Health Organization, Lyon, France, 1986.

- e) United States Food and Drug Administration, *Toxicological principles for the safety of food ingredients and color additives used in food. Draft Redbook II*. 1993.
- f) Rhoades, C.B.; White, R.T., "Mainstream smoke collection by electrostatic precipitation for acid dissolution in a microwave digestion system prior to trace metal determination". *J. AOAC Int.* 80: 1320-1331; 1997.
- g) United States Consumer Product Safety Commission, *Practicability of Developing a Performance Standard to Reduce Cigarette Ignition Propensity*. Washington, D.C.: United States Consumer Product Safety Commission; 1993.
- h) Voncken, P.; Gerstenberg, B.; Rustemeier, K.; Stabbert, R.; Patskan, G.J.; Haussmann, H.J., "Chemical analysis of selected cigarette mainstream smoke constituents for comparative product integrity testing". *Program Booklet and Abstracts 52nd Tobacco Science Research Conference* 52: 36; 1998.
- i) Vorhees, D.J.; Herger-Bernays, W.; McClean, M.D., *Human Health Risk Associated with Cigarette Smoke: The link between smoke constituents and additives*. Report prepared for Mr. Tony Palomba. Menzie-Cura & Associates, Inc.; June 11, 1997.
- j) Vorhees, D.J.; Dodson, R.E., "Estimating risk to cigarette smokers from smoke constituents in proposed "testing and reporting of constituents of cigarette smoke" regulations", Report prepared for the Massachusetts Tobacco Control Program. Menzie-Cura & Associates, Inc.; August 1999.

#### IV. Smoke chemistry

##### a) Mainstream constituents

- 1) Diekmann, J.; Biefel, C.; Stabbert, R.; Rustemeier, K., "Analysis of unsymdimethylhydrazine in cigarette smoke", *Program Booklet and Abstracts 55th Tobacco Science Research Conference*, page 6; 2001.
- 2) Demetriou, D.; Schepers, G., "Determination of nicotine and its metabolites in urine by HPLC after DETBA derivatization", Presented at Biomarkers for Tobacco Exposure: Application to Clinical and Epidemiological Studies, Minneapolis, Minnesota, October 25-26, 2001.
- 3) Federal Trade Commission, "Cigarettes: testing for tar and nicotine content". *Federal Register* 32: 11178; 1967.
- 4) International Organization for Standardization, "Cigarettes - Determination of nicotine content in smoke condensates - gas chromatographic method". *International Organization for Standardization*, ISO 10315: 1989.

- 5) International Organization for Standardization, "Cigarettes - Determination of total and nicotine-free dry particulate matter using a routine analytical smoking machine". *International Organization for Standardization*, 2nd edition, ISO 4387: 1991.
- 6) International Organization for Standardization, "Cigarettes - Determination of carbon monoxide in the vapour phase of cigarette smoke - NDIR method". *International Organization for Standardization*, 2nd edition, ISO 8454: 1995.
- 7) International Organization for Standardization, "Cigarettes; determination of water in smoke condensates - Part 2: Karl Fischer method". *International Organization for Standardization*, ISO 10362-2: 1995.
- 8) Pillsbury, H.C.B.C.C.; O'Conner, K.J.; Irish, F.W., "Tar and Nicotine in cigarette smoke". *Assoc. Off. Anal. Chem.* 52: 458-462; 2002.
- 9) Rustemeier, K.; Patskan, G.; Haussmann, H.J., "The influence of a modified puffing regimen on the yields of smoke constituents from electrically heated and conventional research cigarettes". *The Toxicologist* 54: 16; 2002.
- 10) Rustemeier, K.; Stabbert, R.; Haussmann, H.J.; Roemer, E.; Carmines, E.L., "Evaluation of the potential effects of ingredients added to cigarettes. Part 2: Chemical composition of mainstream smoke". *Food Chem. Toxicol.* 40: 93-104; 2002.
- 11) Stabbert, R.; Schaefer, K.-H.; Biefel, C.; Rustemeier, K., "Analysis of aromatic amines in cigarette". *Program Booklet and Abstracts 55th Tobacco Science Research Conference* 69; 2001. [Prototype]

**b) Sidestream constituents**

**c) ETS constituents**

- 1) Gerstenberg, B.; Stinn, W.; Reininghaus, W., "Comparison of environmental tobacco smoke from an electrically heated and a conventional cigarette". *The Toxicologist* 60: 431; 2001. [Accord E4]
- 2) Gerstenberg, B.; Stinn, W.; Reininghaus, W., "Comparison of environmental tobacco smoke from an electrically heated and a conventional cigarette". *Program Booklet and Abstracts 54th Tobacco Science Research Conference* 19; 2000. [Accord E4]

## V. Biomarkers

### a) Nicotine and metabolites

- 1) Demetriou, D.; Schepers, G., "Determination of nicotine and its metabolites in urine by HPLC after DETBA derivatization", Presented at Biomarkers for Tobacco Exposure: Application to Clinical and Epidemiological Studies, Minneapolis, Minnesota, October 25-26, 2001.
- 2) Demetriou, D.; Schepers, G., "Determination of nicotine and its metabolites in urine by HPLC after DETBA derivatization", Presented at 8<sup>th</sup> Annual Meeting of Society for Research on Nicotine and Tobacco, Savannah, Georgia, February 20-23, 2002.
- 3) Gorrod, J.W.; Schepers, G., "Biotransformation of nicotine and related compounds". In: Gorrod, J.W.; Jacob, P., eds. Analytical determination of nicotine and related compounds and their metabolites Amsterdam: Elsevier Science; 45-67, 1999.
- 4) Rustemeier, K.; Demetriou, D.; Schepers, G.; Voncken, P., "High-performance liquid chromatographic determination of nicotine and its urinary metabolites via their 1,3-diethyl-2-thiobarbituric acid derivatives". *Journal of Chromatography* **613**: 95-103; 1993.
- 5) Schepers, G.; Demetriou, D.; Stabbert, R.; Diehl, B., "Isolation and identification of nicotine phase 2 metabolites from smoker's urine", Presented at the Second Annual Conference of the Society for Research on Nicotine and Tobacco, Washington, D.C., March 15-17, 1996.
- 6) Schepers, G.; Demetriou, D.; Stabbert, R.; Diehl, B.; Seeman, J.I., "5'-hydroxycotinine-N-oxide, a new nicotine metabolite isolated from rat urine". *Xenobiotica* **29**: 793-801; 1999.
- 7) Vanscheeuwijck, P.M.; Teredesai, A.; Terpstra, P.M.; Verbeeck, J.; Kuhl, P.; Gerstenberg, B.; Gebel, S.; Carmines, E.L., "Evaluation of the potential effects of ingredients added to cigarettes. Part 4: subchronic inhalation toxicity". *Food Chem. Toxicol.* **40**: 113-131; 2002.

### b) Carbon monoxide

Klimisch, H.J.; Meissner, K.; Wernicke, H., "Quantitative determination of carbon monoxide in blood by gas chromatography". *Zeitschrift für Klinische Chemie und Klinische Biochemie* **12**: 535-538; 1974.

c) **1,3-Butadiene**

d) **Other**

- 1) Kinser, R.D.; Carchman, R.A.; Leyden, D.E.; Sanders, E.B.; Stabbert, R.; Tricker, A.R.; von Holt, K.; Walk, R.-A., "Selection of biomarkers of exposure for a population study of U.S. adult smokers to cigarette smoke", Presented at Biomarkers for Tobacco Exposure: Application to Clinical and Epidemiological Studies, Minneapolis, Minnesota, October 25-26, 2001.
- 2) Kinser, R.D.; Carchman, R.A.; Leyden, D.E.; Sanders, E.B.; Stabbert, R.; Tricker, A.R.; von Holt, K.; Walk, R.-A., "Selection of biomarkers of exposure for a population study of U.S. adult smokers to cigarette smoke". *Toxicology* 164: 41; 2001.
- 3) Kinser, R.D.; Oey, J., "Examples of systemic validation for biomarkers of cigarette smoke exposure", Presented at Biomarkers for Tobacco Exposure: Application to Clinical and Epidemiological Studies, Minneapolis, Minnesota, October 25-26, 2001.
- 4) Roethig, H.J.; von Holt, K.; Kuhl, P.; McKinney, W.; Zhang, M.; Walk, R.-A., "Biomarkers of potential harm in smokers", Presented at Biomarkers for Tobacco Exposure: Application to Clinical and Epidemiological Studies, Minneapolis, Minnesota, October 25-26, 2001.

V. **Cytotoxicity**

- a) Borenfreund, E.; Puerner, J.A., "Toxicity determined *in vitro* by morphological alterations and neutral red absorption". *Toxicol. Lett.* 24: 119-124; 1985.
- b) Borenfreund, E.; Babich, H.; Martin-Alguacil, N., "Comparisons of two *in vitro* cytotoxicity assays - the neutral red (NR) and tetrazolium MTT tests". *Toxicol. in Vitro* 2: 1-6; 1988.
- c) INVITTOX, "The frame modified neutral red uptake cytotoxicity test", Protocol no. 3a, 1990.
- d) Roemer, E.; Tewes, F.J.; Meisgen, T.J.; Veltel, D.J.; Carmines, E.L., "Evaluation of the potential effects of ingredients added to cigarettes. Part 3: *In vitro* genotoxicity and cytotoxicity". *Food Chem. Toxicol.* 40: 105-111; 2002.

## VI. Mutagenicity (Ames)

- a) Ames, B.N.; McCann, J.; Yamasaki, E., "Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test". *Mutat. Res.* 31: 347-364; 1975.
- b) DeMarini, D.M., "Genotoxicity of tobacco smoke and tobacco smoke condensates". *Mutat. Res.* 114: 59-89; 1983.
- c) Draper, R.; Tewes, F.; Haussmann, H.J., "A comprehensive standardization protocol for the routine biochemical characterization of a rat liver metabolic promutagen activation system used for *in vitro* mutagenicity assays". *Mutagenesis* 5: 619; 1990.
- d) International Conference on Harmonisation, "International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use: Guidance on specific aspects of regulatory genotoxicity tests for pharmaceuticals". *IFPMA*, 38, 1995.
- e) Maron, D.M.; Ames, B.N., "Revised methods for the *Salmonella* mutagenicity test". *Mutat. Res.* 113: 173-215; 1983.
- f) Roemer, E.; Tewes, F.J.; Meisgen, T.J.; Veltel, D.J.; Carmines, E.L., "Evaluation of the potential effects of ingredients added to cigarettes. Part 3: *In vitro* genotoxicity and cytotoxicity". *Food Chem. Toxicol.* 40: 105-111; 2002.
- g) Roemer, E.; Meisgen, T.J.; Tewes, F.J.; Solana, R.P., "Discrimination of cigarette mainstream smoke condensates with the *Salmonella* reverse mutation assay". *Toxicol. Sci.* 42: 295; 1998.
- h) Schramke, H.; Tewes, F.J.; Tewes, F.; Roemer, E., "Using the mouse lymphoma TK assay to compare the mutagenicity of mainstream smoke condensate from research cigarettes", Presented at the 41st annual Meeting of the Society of Toxicology, Nashville, TN, March 17-21, 2002.
- i) Tewes, F.J.; Meisgen, T.J.; Gomm, W.A.; Roemer, E.; Carchman, R.A., "Cigarette parameters that influence the mutagenicity of mainstream smoke condensate". *Toxicol. Sci.* 48: 1999.
- j) Vanschewijck, P.; van Miert, E.; Meurrens, K.; Terpstra, P.M., "The micronucleus assay for the determination of cigarette mainstream smoke activity in bone marrow and peripheral blood of rats". *Toxicol. Sci.* 60: 431; 2001.

## VII. 90-Day rat inhalation

- a) Haussmann, H.-J.; Anskeit, E.; Becker, D.; Kuhl, P.; Stinn, W.; Teredesai, A.; Voncken, P.; Walk, R.-A., "Comparison of fresh and room-aged cigarette sidestream smoke in a subchronic inhalation study on rats". *Toxicological Sciences* **41**: 100-116; 1998.
- b) Kaegler, M.; Teredesai, A.; Vanscheeuwijck, P.; Terpstra, P.; Gerstenberg, B., "Comparison of two exposure regimes in rat subchronic inhalation studies with cigarette smoke". *Toxicol. Sci.* **54**: 17; 2000.
- c) Kaegler, M.; Anskeit, E.; Teredesai, A.; Vanscheeuwijck, P.; Terpstra, P., "Suitability of different exposure regimens in subchronic inhalation studies with cigarette smoke". *Toxicol. Sci.* **60**: 431; 2001.
- d) Organization for Economic Co-operation and Development, "OECD Test Guideline 413, Subchronic inhalation toxicity: 90-day study". OECD Guidelines for testing of chemicals, 5-12-1981.
- e) Organization for Economic Co-operation and Development, "Subchronic inhalation toxicity: 90-day study". OECD Test Guideline 417, 1997.
- f) Roemer, E.; Teredesai, A.; Terpstra, P.; Vanscheeuwijck, P.M.; Haussmann, H.-J., "The discriminative power of rat inhalation studies investigating the irritative property of inhalants", Presented at the Validity of Animal Models of Human Respiratory Disease Santa Fe, NM, September 30- October 2, 1998.
- g) Vanscheeuwijck, P.M.; Teredesai, A.; Terpstra, P.M.; Verbeeck, J.; Kuhl, P.; Gerstenberg, B.; Gebel, S.; Carmines, E.L., "Evaluation of the potential effects of ingredients added to cigarettes. Part 4: subchronic inhalation toxicity". *Food Chem. Toxicol.* **40**: 113-131; 2002.

## VIII. Smoking topography

- a) United States Consumer Product Safety Commission, Toxicity testing plan for low ignition-potential cigarettes. In: Lee, B.C.; Burns, D.M.; Gairola, C.G.; Harris, J.E.; Hoffman, D.; Pillsbury, H.C., Jr.; Coplanar, DR, eds. Toxicity Testing Plan Washington, D.C.: United States Consumer Product Safety Commission; Volume 5 Part 1, 1993.
- b) Kinser, R.D.; Carchman, R.A.; Leyden, D.E.; Sanders, E.B.; Stabbert, R.; Tricker, A.R.; von Holt, K.; Walk, R.-A., "Selection of biomarkers of exposure for a population study of U.S. adult smokers to cigarette smoke", Presented at Biomarkers for Tobacco Exposure: Application to Clinical and Epidemiological Studies, Minneapolis, Minnesota, October 25-26, 2001.

- c) Kinser, R.D.; Carchman, R.A.; Leyden, D.E.; Sanders, E.B.; Stabbert, R.; Tricker, A.R.; von Holt, K.; Walk, R.-A., "Selection of biomarkers of exposure for a population study of U.S. adult smokers to cigarette smoke". *Toxicology* 164: 41; 2001.
- d) Kinser, R.D.; Oey, J., "Examples of systemic validation for biomarkers of cigarette smoke exposure", Presented at Biomarkers for Tobacco Exposure: Application to Clinical and Epidemiological Studies, Minneapolis, Minnesota, October 25-26, 2001.
- e) Walk, R.-A.; Carter, W.H.; Carchman, R.A.; Gear, J.C.; Gomm, W.; Gennings, C.; Kinser, R.D.; Leyden, D.E.; Nelson, B.L.; Tricker, A.R.; von Holt, K., "Design for a population study of exposure of U.S. adult smokers to cigarette smoke". *Toxicology* 164: 41; 2001.
- f) Walk, R.-A.; Carter, W.H.; Carchman, R.A.; Gear, J.C.; Gomm, W.; Gennings, C.; Kinser, R.D.; Leyden, D.E.; Nelson, B.L.; Tricker, A.R.; von Holt, K., "Design for a population study of exposure of U.S. adult smokers to cigarette smoke". Presented at Biomarkers for Tobacco Exposure: Application to Clinical and Epidemiological Studies, Minneapolis, Minnesota, October 25-26, 2001.

#### **IX. Questionnaires for human exposure/risk evaluation**

#### **X. Short-term confined clinical design**

#### **XI. Surveillance plan**

#### **XII. General methods**

- a) American Association for Laboratory Animal Science, "American Association for Laboratory Animal Science Policy on the humane care and use of laboratory animals". *Lab. Anim. Sci.* 41: 91; 1991. [Animal care]
- b) Dunnett, C.W., "A multiple comparison procedure for comparing several treatments with a control". *J. Am. Stat. Assoc.* 50: 1096-1121; 1955. (Statistics)
- c) Mann, H.B.; Whitney, D.K., "On a test of whether one of two random variables is stochastically larger than the other". *Annals of Mathematical Statistics* 18: 50-60; 1947. [Statistics]
- d) Sachs, L., *Applied Statistics*. New York: Springer Verlag; 1982. (Statistics)
- e) International Organization for Standardization., "Tobacco and tobacco products -- Atmosphere for conditioning and testing", *International Organization for Standardization*, 3rd edition, (ISO 3402): 1991. [Cigarette conditioning]

- f) Organization for Economic Co-operation and Development, "GLP. Consensus document: The OECD principles of good laboratory practice". 1992. [Good laboratory practices]
- g) International Agency for Research on Cancer, "Overall evaluations of carcinogenicity to humans". [Online] <http://193.51.164.11/monoeval/crthall.html> Accessed 2000. [Carcinogenicity classification]
- h) Diana, J.N.; Vaught, A. "Research cigarettes". The university of Kentucky Printing Services, Lexington, KY. 1990. [Reference cigarettes]
- i) Reininghaus, W.; Hackenberg, U., "Anlage zur Langzeit-inhalation mit Zigarettenrauch für kleine Labortiere". *Medizin Technik* 97: 5-6; 1977. [Smoking machine]

### Submitted Manuscripts for Tracking

1. Kinser, R.D.; Nelson, B.L.; Roethig, H.J.; Walk, R.-A.; Oey, J.; Tricker, A.R.; Leyden, D.E., "Assessment of human exposure to cigarette smoke constituents; Pilot study results for carbon monoxide". Accepted for presentation at Society of Toxicology meeting, Nashville, Tennessee, March 17-21, 2002. [CO Biomarkers, *in-vivo* (Human) studies]
2. Roethig, H.J.; Oey, J.; Nelson, B.N.; Walk, R.A., "Dose-response investigations of carboxy-hemoglobin of adult smokers smoking conventional and electrically heated cigarettes". To be presented at the Society of Toxicology annual meeting, Nashville, Tennessee, March 17-21, 2002. [CO biomarkers, *in-vivo* (Human) Studies, E4]
3. Roethig, H.J.; Oey, J.; Nelson, B.L.; Walk, R.A., "Investigation of carboxy-hemoglobin of adult smokers of conventional cigarettes and an electrically heated cigarette (Oasis)", To be presented at the 29th Annual Meeting of the Japanese Society of Toxicology, Nagoya Congress Center, Japan, June 18-20, 2002. [CO biomarkers, *in-vivo* (Human) Studies, E4]